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US006461617B1

(12) **United States Patent**
Shone et al.

(10) **Patent No.: US 6,461,617 B1**
(45) **Date of Patent: Oct. 8, 2002**

(54) **RECOMBINANT TOXIN FRAGMENTS**

(75) Inventors: **Clifford Charles Shone**; **Conrad Padraig Quinn**, both of Wiltshire; **Keith Alan Foster**, Surrey, all of (GB)

(73) Assignees: **Microbiological Research Authority**, Salisbury (GB); **The Speywood Laboratory Limited**, London (GB)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/255,829**

(22) Filed: **Feb. 23, 1999**

Related U.S. Application Data

(63) Continuation of application No. PCT/GB97/02273, filed on Aug. 22, 1997, which is a continuation-in-part of application No. 08/782,893, filed on Dec. 27, 1996, now abandoned.

(30) **Foreign Application Priority Data**

Aug. 23, 1996 (GB) 9617671
Dec. 13, 1996 (GB) 9625996

(51) **Int. Cl.**[7] **A61K 39/02**; A61K 39/38; A61K 39/00; C12P 21/06; C12P 21/04

(52) **U.S. Cl.** **424/236.1**; 424/157.1; 424/164.1; 424/167.1; 424/178.1; 424/179.1; 424/184.1; 424/235.1; 424/234.1; 424/236.1; 424/239.1; 424/247.1; 530/300; 530/350; 530/825; 435/69.1; 435/70.1; 435/71.1; 435/71.2; 435/69.7; 435/252.33; 536/23.4; 536/23.7

(58) **Field of Search** 424/236.1, 235.1, 424/234.1, 184.1, 237.1, 247.1, 183.1, 178.1, 179.1; 530/300, 350, 825; 426/167.1, 164.1, 157.1; 435/69.1, 70.1, 71.1, 71.2, 69.7, 252.35, 320.11; 536/23.7, 23.4

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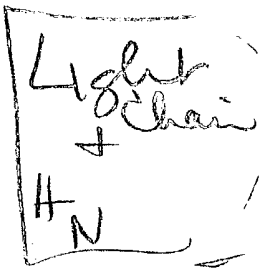

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(57) **ABSTRACT**

A polypeptide has first and second domains which enable the polypeptide to be translocated into a target cell or which increase the solubility of the polypeptide, or both, and further enable the polypeptide to cleave one or more vesicle or plasma-membrane associated proteins essential to exocytosis. The polypeptide thus combines useful properties of a clostridial toxin, such as a botulinum or tetanus toxin, without the toxicity associated with the natural molecule. The polypeptide can also contain a third domain that targets it to a specific cell, rendering the polypeptide useful in inhibition of exocytosis in target cells. Fusion proteins comprising the polypeptide, nucleic acids encoding the polypeptide and methods of making the polypeptide are also provided. Controlled activation of the polypeptide is possible and the polypeptide can be incorporated into vaccines and toxin assays.

10 Claims, 11 Drawing Sheets

L3: Entry 16 of 49 File: USPT Oct 8, 2002

US-PAT-NO: 6461617
DOCUMENT-IDENTIFIER: US 6461617 B1
**** See image for Certificate of Correction ****

TITLE: Recombinant toxin fragments

DATE-ISSUED: October 8, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Shone; Clifford Charles	Wiltshire			GB
Quinn; Conrad Padraig	Wiltshire			GB
Foster; Keith Alan	Surrey			GB

US-CL-CURRENT: 424/236.1; 424/157.1, 424/164.1, 424/167.1, 424/178.1, 424/179.1, 424/184.1, 424/234.1, 424/235.1, 424/239.1, 424/247.1, 435/252.33, 435/69.1, 435/69.7, 435/70.1, 435/71.1, 435/71.2, 530/300, 530/350, 530/825, 536/23.4, 536/23.7

CLAIMS:

What is claimed is:

1. A non-toxic polypeptide comprising first, second and third domains, wherein (a) said first domain comprises a botulinum toxin light chain and cleaves one or more vesicle or plasma-membrane associated proteins essential to exocytosis, (b) said second domain comprises the first 100 N-terminal amino acids of a botulinum toxin heavy chain and (i) translocates the polypeptide into a cell or (ii) increases the solubility of the polypeptide compared to the solubility of the first domain on its own or (iii) both translocates the polypeptide into a cell and increases the solubility of the polypeptide compared to the solubility of the first domain on its own, (c) said polypeptide is free of clostridial neurotoxin and free of clostridial neurotoxin precursor that can be converted into toxin by proteolytic action, (d) said polypeptide is a single polypeptide, (e) said third domain is a tandem repeat synthetic IgG binding domain derived from domain .beta. of Staphylococcal protein A, and (f) said polypeptide lacks a portion designated H.sub.c of a botulinum toxin heavy chain.

2. A non-toxin polypeptide comprising first, second and third domains, wherein (a) said first domain comprises a botulinum toxin light chain and cleaves one or more vesicle or plasma-membrane associated proteins essential to exocytosis, (b) said second domain comprises the first 100 N-terminal amino acids of a botulinum toxin heavy chains and (i) translocates the polypeptide into a cell of (ii) increases the solubility of the polypeptide compared to the solubility of the first domain on its own or (iii) both translocates the polypeptide into a cell and increases the solubility of the polypeptide compared to the solubility of the first domain on its own, (c) said polypeptide is free of clostridial neurotoxin and free of clostridial neurotoxin precursor that can be converted into toxin by proteolytic action, (d) said polypeptide is a single polypeptide, (e) said third domain is insulin-like growth-factor-1 (IGF-1), and (f) said polypeptide lacks a portion

designated H.sub.c of a botulinum toxin heavy chain.

3. A non-toxin polypeptide comprising first and second domains, wherein (a) said first domain is a botulinum toxin type A light chain variant comprising a sequence correspond to amino acids 1-448 of SEQ ID NO:2 wherein three amino acid residues have been altered compared to that sequence, namely at residue 2 a glutamate, at residue 26 a lysine and at residue 27 a tyrosine which first domain cleaves one or more vesicle or plasma-membrane associated proteins essential to exocytosis, (b) said second domain comprises the first 100 N-terminal amino acids of a botulinum toxin heavy chains and (i) translocates the the polypeptide into a cell of (ii) increases the solubility of the polypeptide compared to the solubility of the first domain on its own or (iii) both translocates the polypeptide into a cell and increases the solubility of the polypeptide compared to the solubility of the first domain on its own, (c) said polypeptide is free of clostridial neurotoxin and free of clostridial neurotoxin precursor that can be converted into toxin by proteolytic action, (d) said polypeptide is a single polypeptide, (e) one or both of (i) the toxin light chain or fragment or variant of toxin light chain and (ii) the portion of the toxin heavy chain are of botulinum toxin type A, and (f) said polypeptide lacks a portion designated H.sub.c of a botulinum toxin heavy chain.

4. A non-toxin polypeptide comprising first and second domains, wherein (a) first domain comprises a botulinum toxin light chain and cleaves one or more vesicle or plasma-membrane associated proteins essential to exocytosis, (b) said second domain comprises the first 100 N-terminal amino acids of a botulinum toxin heavy chain and (i) translocates the polypeptide into a cell or or (ii) increases the solubility of the polypeptide compared to the solubility of the first domain on its own or (iii) both translocates the polypeptide into a cell and increases the solubility of the polypeptide compared to the solubility of the first domain on its own, (c) said polypeptide is free of clostridial neurotoxin and free of clostridial neurotoxin precursor that can be be converted into toxin by proteolytic action, (d) said polypeptide is a single single polypeptide, and (e) said polypeptide lacks a portion designated H.sub.c of a botulinum toxin heavy chain.

5. A non-toxin polypeptide comprising first and second domains, wherein (a) said first domain comprises a botulinum toxin light chain and cleaves one or more vesicle or plasma-membrane associated proteins essential to exocytosis, (b) said second domain comprises the first 100 N-terminal amino acids of a botulinum toxin heavy chain and (i) translocates the polypeptide into a cell or or (ii) increases the solubility of the polypeptide compared to the solubility of the first domain on its own or (iii) both translocates the polypeptide into a cell and increases the solubility of the polypeptide compared to the solubility of the first domain on its own, (c) said polypeptide is free of clostridial neurotoxin and free of clostridial neurotoxin precursor that can be be converted into toxin by proteolytic action, (d) said polypeptide is a single single polypeptide, (e) said second domain comprises a portion designated H.sub.N of the botulinum toxin heavy chain which consists of the 423 N-terminal amino acids of a botulinum toxin type A heavy chain, and (f) said polypeptide lacks a portion designated H.sub.c of a botulinum toxin heavy chain.

6. A polypeptide according to claim 5 wherein the first domain comprises a botulinum toxin type A light chain.

7. A polypeptide according to claim 5 wherein said first domain is a botulinum toxin type A light chain variant which comprises a sequence corresponding to amino acids 1-448 of SEQ ID NO:2 having at least three amino acid residues

which are altered compared to that sequence, namely at residue 2 a glutamate, residue 26 a lysine and residue 27 a tyrosine, and wherein said polypeptide contains 423 N-terminal amino acids of a botulinum toxin type A heavy chain.

8. A polypeptide comprising a botulinum toxin light chain and a botulinum toxin toxin heavy chain lacking a C-terminal part of the botulinum toxin heavy chain designated H.sub.c wherein said botulinum toxin heavy chain is not capable of binding to cell surface receptors.

9. A polypeptide according to claim 8 wherein said heavy chain lacks amino acid residues 872 -1296 of botulinum toxin A.

10. A polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO. 2, 4, 6, 10, 12, 14, 16, 18, 20, 22, 24, and 26.

US006287566B1

(12) **United States Patent**
Dertzbaugh

(10) **Patent No.: US 6,287,566 B1**
(45) **Date of Patent: Sep. 11, 2001**

(54) **PROTECTIVE PEPTIDES NEUROTOXIN OF *C. BOTULINUM***

(75) Inventor: **Mark T. Dertzbaugh**, Frederick, MD (US)

(73) Assignee: **The United States of America as represented by the Secretary of the Army**, Washington, DC (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **08/446,114**

(22) Filed: **May 19, 1995**

(51) **Int. Cl.**[7] **A61K 39/00**; A61K 39/02; A61K 39/08

(52) **U.S. Cl.** **424/190.1**; 424/192.1; 424/239.1; 530/300; 530/350; 930/200

(58) **Field of Search** 424/236.1, 239.1, 424/247.1, 184.1, 185.1, 190.1, 192.1; 530/350, 300; 930/10, 200

(56) **References Cited**

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Primary Examiner—Patricia A. Duffy
(74) *Attorney, Agent, or Firm*—Elizabeth Arwine; John Francis Moran; Charles H. Harris

(57) **ABSTRACT**

Methods for developing vaccines to protect from neurotoxins of *C. botulinum* have been developed. Truncated BoNT/A proteins of about 15–30 kDa in size produced immune responses that provided protection from neuronal damage by *botulinum* neurotoxins.

5 Claims, No Drawings

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L4: Entry 73 of 148 File: USPT Sep 11, 2001

US-PAT-NO: 6287566
DOCUMENT-IDENTIFIER: US 6287566 B1

TITLE: Protective peptides neurotoxin of C. botulinum

DATE-ISSUED: September 11, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Dertzbaugh; Mark T.	Frederick	MD		

US-CL-CURRENT: 424/190.1; 424/192.1, 424/239.1, 530/300, 530/350, 930/200

CLAIMS:

What is claimed is:

1. An isolated polypeptide consisting of at least 100 amino acids from either sequence

H.sub.3 H-IKVNN WDLFF SPSED NFTND LNKGE EITSD TNIEA AEENI SLDLI QQYYL TFNFD NEPEN ISIEN LSSDI IGQLE LMPNI ERFPN GKKYE LDKYT MFHYL RAQEF EHGKS RIALT NSVNE ALLNP SRVYT FFSSD YVKKV NKATE AAMFL GWVEQ LVYDF TDETS EVSTT DKIAD ITIII PYIGP ALNIG NMLYK DDFVG ALIFS GA-COOH (Seq. ID No. 21)

or

H.sub.3 N-LNSSL YRGTK FIIKK YASGN KDNIV RNNDR VYINV VVKNK EYRLA TNASQ AGVEK ILSAL EIPDV GNLSQ VVVMK SKNDQ GITNK CKMNL QDNNG NDIGF IGFHQ FNNIA KLVAS NWYNR QIERS SRTLG CSWEF IPVDD-COOH (Seq. ID NO. 22).

2. A composition of matter comprising at least one polypeptide of claim 1 in a carrier.

3. An isolated fusion protein of wherein a first polypeptide consisting of at least 100 amino acids is from either sequence

H.sub.3 H-IKVNN WDLFF SPSED NFTND LNKGE EITSD TNIEA AEENI SLDLI QQYYL TFNFD NEPEN ISIEN LSSDI IGQLE LMPNI ERFPN GKKYE LDKYT MFHYL RAQEF EHGKS RIALT NSVNE ALLNP SRVYT FFSSD YVKKV NKATE AAMFL GWVEQ LVYDF TDETS EVSTT DKIAD ITIII PYIGP ALNIG NMLYK DDFVG ALIFS GA-COOH (Seq. ID NO. 21)

or

H.sub.3 N-LNSSL YRGTK FIIKK YASGN KDNIV RNNDR VYINV VVKNK EYRLA TNASQ AGVEK ILSAL EIPDV GNLSQ VVVMK SKNDQ GITNK CKMNL QDNNG NDIGF IGFHQ FNNIA KLVAS NWYNR

QIERS SRTLG CSWEF IPVDD-COOH (Seq. ID NO. 22) is fused to a second polypeptide which acts as an adjuvant.

4. A polypeptide of claim 3 wherein the second polypeptide is 2A polypeptide of of cholera toxin.

5. A method of immunizing a mammal susceptible to botulism by administration of of a composition of claim 2.

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US006270777B1

(12) **United States Patent**
Sokol et al.

(10) **Patent No.: US 6,270,777 B1**
(45) **Date of Patent: Aug. 7, 2001**

(54) **CONSERVED METALLOPROTEASE EPITOPES**

(75) Inventors: **Pamela A. Sokol**; **Cora D. Kooi**, both of Calgary (CA)

(73) Assignee: **University Technologies International Inc.** (CA)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **08/772,282**

(22) Filed: **Dec. 20, 1996**

(51) **Int. Cl.**[7] **A61K 39/104**; A61K 39/02; C07K 14/195; C07K 14/21

(52) **U.S. Cl.** **424/260.1**; 424/184.1; 424/190.1; 424/234.1; 424/130.1; 424/94.67; 424/261.1; 424/246.1; 424/185.1; 424/197.11; 530/300; 530/324; 530/325; 530/326; 530/327; 530/328; 530/350; 530/387.1

(58) **Field of Search** 424/234.1, 260.1, 424/261.1, 246.1, 190.1, 184.1, 185.1, 197.11, 130.1, 94.67; 530/300, 324–328, 350, 387.1; 514/2

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(57) **ABSTRACT**

The present invention provides immunogenic compositions comprising peptides derived from the amino acid sequence of *P. aeruginosa* for protecting susceptible hosts against diseases caused by bacterial pathogens which secrete a zinc metalloprotease.

15 Claims, 5 Drawing Sheets

L3: Entry 25 of 49 File: USPT Aug 7, 2001

US-PAT-NO: 6270777
DOCUMENT-IDENTIFIER: US 6270777 B1

TITLE: Conserved metalloprotease epitopes

DATE-ISSUED: August 7, 2001

INVENTOR-INFORMATION:

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US-CL-CURRENT: 424/260.1; 424/130.1, 424/184.1, 424/185.1, 424/190.1, 424/197.11, 424/234.1, 424/246.1, 424/261.1, 424/94.67, 530/300, 530/324, 530/325, 530/326, 530/327, 530/328, 530/350, 530/387.1

CLAIMS:

We claim:

1. A peptide consisting of the amino acid sequence VSHGFTEQNSGLIYRGQSGGMNEAF (Sequence ID No: 1) or a fragment or conservative amino acid substitution variant thereof, which contains an epitope which is recognized by an antibody which neutralizes the proteolytic activity of Pseudomonas aeruginosa.

2. The peptide or fragment or variant of claim 1 wherein the peptide or fragment or variant is a peptide having an amino acid sequence comprising at least nine consecutive amino acids from the amino acid sequence VSHGIFTEQNSGLIYRGQSGGMNEAF (Sequence ID No: 1).

3. The peptide or fragment or variant of claim 1 wherein the peptide or fragment or variant is a fragment selected from the group consisting of

(a) VSIIGPTEQN (Sequence ID No:2);

(b) HGFTEQNSG (Sequence ID No:3);

(c) FTEQNSGLI (Sequence ID No:4);

(d) EQNSGLIYR (Sequence ID No:5);

(e) NSGLIYRGQ (Sequence ID No:6);

(f) GLIYRGQSG (Sequence ID No:7);

(g) IYRGQSGGM (Sequence ID No:8);

(h) RGQSGGMNE (Sequence ID No:9);

(i) QSGGMNEAF (Sequence ID No:10).

4. A peptide consisting of the amino acid sequence HGFTEQNSG (Sequence ID No:3).

5. A peptide consisting of the amino sequence SGALRYMDQPSRDGRSIDM (Sequence ID No: 11) or a fragment or conservative amino acid substitution variant thereof, which contains an eptitope which is recognized by an antibody which neutralizes neutralizes the proteolytic activity of Pseudomonas aeruginosa.

6. The peptide or fragment or variant of claim 5 wherein the peptide or fragment or variant is a peptide having an amino acid sequence comprising at least nine consecutive amino acids from the amino acid sequence SGALRYMDQPSRDGRSIDM (Sequence ID No: 11).

7. The peptide or fragment or variant of claim 5 wherein the peptide or fragment or variant is a fragment selected from the group consisting of

(a) SGALRYMDQ (Sequence ID No: 12);

(b) ALRYMDQPS (Sequence ID No: 13);

(c) RYMDQPSRD (Sequence ID No:14);

(d) MDQPSRDGR (Sequence ID No:15);

(e) QPSRDGRSI (Sequence ID No: 16);

(f) SRDGRSIDM (Sequence ID No: 17).

8. A peptide consisting of the amino acid sequence RYMDQPSRD (Sequence IN No:14).

9. An immunogenic composition comprising at least one active component selected from the group consisting of:

(a) a peptide consisting of the amino acid sequence HGFTEQNSG (Sequence ID No:3);

(b) a peptide consising of the amino acid sequence RYMDQPSRD (Sequence ID No:4);

(c) a peptide consisting of the amino acid sequence VSHGFTEQNSGLIYRGQSGGMNEAF (Sequence ID No:1);

(d) a peptide consisting of the amino acid sequence SGALRYMDQPSRIDGRSIDM (Sequence ID No.:11); and

(e) a fragment or conservative amino acid substitution variant of a peptide of (a), (b), (c) or (d), which contains a epitope which is recognized by an antibody which neutralizes the proteolytic activity of Pseudomonas aeruginosa

and a pharmaceutically acceptable carrier, said at least one active component producing an immune response when administered to a host.

10. The immunogenic composition of claim 9 formulated as a vaccine for administration to a mammal to protect the mammal against a disease caused by a bacterial pathogen which secretes a zinc metalloprotease.

11. The immunogenic composition of claim 10 wherein the bacterial pathogen secretes a thermolysin-like metalloprotease.

12. The inmmunogenic composition of claim 10 wherein the mammal is to be protected against a disease caused by a pathogen selected from the group consisting of Pseudomonas aeruginosa, B. cepacia, Vibrio cholerae, V. vulnificus, Legionella pneumophila, Serratia marcescens, Bacillus anthracis, Clostridium tetani, Clostridium botulinum, Aeromonas hydrophilia, Staphylococcus epidermidis, Staphylococcus aureus, Streptococcus sanguis, Streptococcus faecalis, Lysteria monocytogenes, and Pasteurella haemolytica.

13. The immunogenic composition of claim 12 wherein the at least one active component is selected from the group consisting of

(a) the peptide HGFTEQNSG (Sequence ID No:3);

(b) the peptide RYMDQPSRD (Sequence ID No:14);

(c) a mixture of the peptide HGFTEQNSG (Sequence ID No:3), and the peptide RYMDQPSRD (Sequence ID No:14).

14. The immunogenic composition of claim 13 wherein the peptide is conjugated to a carrier protein.

15. The immunogenic composition of claim 14 wherein the carrier protein is selected from the group consisting of keyhole limpet haemocyanin, diphtheria toxoid, diphtheria toxin CRM197, tetanus toxoid, P. aeruginosa exotoxin A mutant form, cholera toxin B subunit, pertussis toxin subunits, measles virus F protein and Haemophilus PRP outer membrane protein.

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(12) **United States Patent**
Johnston et al.

(10) **Patent No.: US 6,521,235 B2**
(45) **Date of Patent: *Feb. 18, 2003**

(54) **ALPHAVIRUS RNA REPLICON SYSTEMS**

(75) Inventors: **Robert E. Johnston**, Chapel Hill, NC (US); **Nancy L. Davis**, Chapel Hill, NC (US); **Jonathan F. Smith**, Cary, NC (US); **Peter Pushko**, Frederick, MD (US); **Michael Parker**, Frederick, MD (US); **George Ludwig**, Frederick, MD (US)

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

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(65) **Prior Publication Data**

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(52) **U.S. Cl.** **424/199.1**; 424/218.1; 435/235.1; 435/236; 435/320.1

(58) **Field of Search** 435/235.1, 236, 435/320.1; 424/199.1, 218.1

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(57) **ABSTRACT**

The present invention provides a helper cell for expressing an infectious, replication defective, alphavirus particle in an alphavirus-permissive cell. The helper cell includes (a) a first helper RNA encoding (i) at least one alphavirus structural protein, and (ii) not encoding at least one alphavirus structural protein; and (b) a second helper RNA separate from the first helper RNA, the second helper RNA (i) not encoding the alphavirus structural protein encoded by the first helper RNA, and (ii) encoding the at least one alphavirus structural protein not encoded by the first helper RNA. Preferably, the helper cell is co-transfected with a replicon RNA encoding an alphavirus packaging segment and an inserted heterogeneous RNA, such that all of the alphavirus structural proteins assemble together into alphavirus particles in the cell, with said replicon RNA packaged therein.

78 Claims, 2 Drawing Sheets

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(12) **United States Patent**
Sachs et al.

(10) **Patent No.: US 6,776,990 B2**
(45) **Date of Patent: Aug. 17, 2004**

(54) **METHODS AND COMPOSITIONS FOR THE TREATMENT OF PANCREATITIS**

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(73) Assignee: **Allergan, Inc.**, Irvine, CA (US)

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(58) **Field of Search** 514/2, 12; 424/192.1, 424/193.1, 94.1

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(57) **ABSTRACT**

Methods and compositions for the treatment of acute pancreatitis in a mammal. Particular compositions comprise a binding element, a translocation element, and a therapeutic element able to prevent accumulation of digestive enzymes within the pancreas.

24 Claims, No Drawings

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☐ 50. 5443966. 23 Aug 93; 22 Aug 95. Expression of tetanus toxin fragment C. Fairweather; Neil F., et al. 435/69.3; 435/252.33 435/320.1 435/69.1. C12P021/02 C12N015/70 C12N001/21.

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Terms	Documents
L1 and (botx or bota or botox or botulin or botulism or botulinum or toxin or neurotoxin or bont or rbotox or rbonta).clm.	

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L2: Entry 27 of 58 File: USPT Jul 6, 1999

DOCUMENT-IDENTIFIER: US 5919665 A
TITLE: Vaccine for clostridium botulinum neurotoxin

CLAIMS:

1. A soluble fusion protein comprising a non-toxin protein sequence and a portion of of the Clostridium botulinum type A toxin, said portion of the Clostridium botulinum type A toxin comprising a portion of the sequence of SEQ ID NO:28.

2. The fusion protein of claim 1, wherein said portion of the Clostridium botulinum type A toxin sequence comprises SEQ ID NO:23.

3. The fusion protein of claim 1, wherein said non-toxin protein sequence comprises a poly-histidine tract.

6. A host cell containing a recombinant expression vector, said vector encoding a protein comprising at least a portion of a Clostridium botulinum type A toxin protein sequence of SEQ ID NO:28, and wherein said host cell is capable of expressing said protein as a soluble protein in said host cell at a level greater than or equal to 0.75% of the total cellular protein.

7. The host cell of claim 6, wherein said portion of a toxin comprises SEQ ID NO:23.

10. A soluble fusion protein, comprising at least a portion of Clostridium botulinum C fragment linked to a poly-histidine tag.

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L2: Entry 12 of 58 File: USPT Feb 18, 2003

DOCUMENT-IDENTIFIER: US 6521235 B2
TITLE: Alphavirus RNA replicon systems

CLAIMS:

77. The composition of claim 51, wherein the immunogen is a Botulinum toxin C fragment immunogen.

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